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(54) Title: RECEPTOR PROTEINS

(57) Abstract: A method of determining whether an individual is predisposed to inflammatory bowel disease, which method comprises identifying whether the individual has a polymorphism in the CCRL2 polynucleotide or protein which polymorphism is associated with inflammatory bowel disease.

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RECEPTOR PROTEINS

Field of the Invention

The present invention relates to a method of determining whether an individual is predisposed to inflammatory bowel disease and to associated methods, uses, agents, compounds and compositions.

Background to the Invention

It is now recognised that there is considerable genetic diversity in human populations, with common polymorphisms occurring on average at least every thousand bases in the genome. A polymorphism is generally understood to mean a sequence variation which is present at a reasonable frequency in the population. In contrast a mutation is a rare deleterious sequence variation. Each form of sequence is termed an allele. In reality, the distinction between polymorphisms and mutations is artificial, as there is a continuum of allele frequencies from mutations present in a single individual to pairs of common alleles present in 50% of the population. The functional impact of these allelic variants is likewise a continuum, with some alleles conferring no detectable functional effects, whilst others completely abrogate gene function. In general, alleles that exert major deleterious effects on gene function are likely to be rare, unless there is some selective pressure to maintain them in populations.

In this application, we will use the term polymorphism to mean an allelic variant, irrespective of its frequency in the population. These polymorphisms, where they affect gene expression or activity of the encoded gene product, must therefore account for a significant percentage of disease susceptibility, either directly or through interaction with other genetic and environmental factors.

A major objective of the current drive to sequence the human genome and characterise common polymorphism is to understand better the molecular basis for disease. Not only will this lead to new and improved targets for drugs, but it will also allow better matching of treatment and disease, and provide assays for genetic risk factors that identify those individuals that are at greatest risk of disease. This would allow both screening and treatment to be better targeted, increasing the cost-effectiveness of health-care provision.

One area in which new approaches are badly needed is in the treatment of Inflammatory Bowel Disease (IBD). Genetic factors that influence susceptibility to this

disorder offer the prospect of providing important new insights, and will yield important new diagnostic and prognostic tests.

Chemokines acting through their cognate receptors are critical for the recruitment of effector immune cells to inflamed tissues, and are therefore of considerable interest as potential targets for the treatment of inflammatory disease. CCRL2 (also known as HCR) encodes a chemokine receptor-like protein, which is predicted to be a seven transmembrane protein and most closely related to the G protein coupled receptor CCR1. G protein coupled receptors (GPCRs) are a family of approximately 500 proteins with a 7 transmembrane structure that transduce cellular signals of a variety of biological mediators. The interaction of a GPCR and its ligand causes a conformational change in the protein and facilitates the binding of small associated heterotrimeric G proteins to the intracellular receptor domains which initiate a signalling cascade. GPCRs are cell surface receptors and therefore are attractive targets for pharmacological intervention. CCRL2 is expressed at high levels in primary neutrophils and primary monocytes, and is further upregulated on neutrophil activation and when monocytes differentiate to macrophages. However, the importance of CCRL2 in human inflammatory disease has not been investigated.

Summary of the Invention

The current invention identifies CCRL2 as a gene that is highly expressed in colonic tissue from patients with ulcerative colitis. Independently, in a genome-wide search for SNPs in GPCR genes we identified a missense SNP (CCRL2-204) and a silent SNP (CCRL2-202) in the gene for CCRL2. We subsequently discovered that these variants are significantly associated with the development of ulcerative colitis. Thus we have unambiguously identified an important role for this gene in modulating monocyte/macrophage function, and in the pathophysiology of IBD. This finding leads directly to new ways of predicting susceptibility to IBD, diagnosis and prognosis of the disease, selection of appropriate treatment regimes, and new approaches to IBD treatment involving the modulation of monocyte/macrophage behaviour through the use of agonists, antagonists and inverse agonists of CCRL2 activity.

The present invention identifies polymorphisms in the human CCRL2 genomic region which concerns an increased risk of developing inflammatory bowel disease. The identification of new allelic variants of the CCRL2 gene may affect CCRL2 activity both indirectly, through effects on mRNA structure and expression, and directly by altering the amino acid sequence of the receptor. Analysis of an individual's DNA or protein for the

presence of a particular polymorphism allows the diagnosis of CCRL2 -mediated inflammatory bowel disease and indicates the use of a CCRL2 modulator to treat said condition.

Accordingly, the present invention provides:

- a method of determining whether an individual is predisposed to inflammatory bowel disease, which method comprises identifying whether the individual has a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease;
- a method for preventing or treating an individual who has been identified as having a predisposition to inflammatory disease, by a method according to the invention, comprising administering to the individual a therapeutically effective amount of an agent which prevents or treats inflammatory bowel disease;
- the use of an agent which prevents or treats inflammatory bowel disease, in the manufacture of a medicament for use in preventing or treating an individual, which individual has been identified as having a predisposition to inflammatory bowel disease;
- a method for identifying an agent for the prevention or treatment of inflammatory bowel disease, the method comprising:
  - (i) contacting a test agent with a CCRL2 polypeptide or polynucleotide having a mutation or polymorphism associated with inflammatory bowel disease; and
  - (ii) determining whether the test agent is capable of binding to the polypeptide or polynucleotide and modulating the activity or expression of the polypeptide or polynucleotide;
- a method of formulating a pharmaceutical composition comprising:
  - (i) identifying an agent for the prevention or treatment of inflammatory bowel disease by a method of the invention; and
  - (ii) formulating the agent with a pharmaceutically acceptable carrier or diluent;
- an agent identified by a method of the invention;
- a pharmaceutical composition for the prevention or treatment of inflammatory bowel disease comprising an agent according to the invention and a pharmaceutically acceptable carrier or diluent;
- use of an agent according to the invention in the manufacture of a medicament for use in treating or preventing inflammatory bowel disease;
- a method of preventing or treating inflammatory bowel disease, which method

comprises administering a therapeutically effective amount of an agent according to the invention to an individual in need thereof;

- a method of treating inflammatory bowel disease, which method comprises:
  (i) identifying an agent for the prevention or treatment of inflammatory bowel disease by a method according to the invention; and
  (ii) administering a therapeutically effective amount of an agent identified in (i) to an individual having inflammatory bowel disease.
- a probe, primer or antibody for use in a method according to the invention which is capable of selectively detecting a polymorphism in the CCRL2 polynucleotide or protein associated with inflammatory bowel disease (i.e. the probe, primer or antibody is generally capable of selectively detecting a CCRL2 polynucleotide or protein that comprises the polymorphism);
- an antibody capable of selectively binding to a polymorphism associated with inflammatory bowel disease in the CCRL2 protein;
- a method of identifying a polymorphism which can be used to determine whether an individual has a genetic predisposition to inflammatory bowel disease, the method comprising screening the CCRL2 polynucleotide or polypeptide of one or more individuals;
- a method for treating or preventing inflammatory bowel disease in an individual comprising:
  (i) identifying, in the individual, the presence of a polymorphism in the CCRL2 polynucleotide or CCRL2 protein which is associated with inflammatory bowel disease; and
  (ii) introducing into the individual a CCRL2 polynucleotide of the invention which is a different allele of the polymorphism or polymorphisms to that present in the individual;
- a cell line comprising a CCRL2 polynucleotide having a polymorphism associated with inflammatory bowel disease;
- a non-human animal which is transgenic for a CCRL2 polynucleotide having a polymorphism associated with inflammatory bowel disease;
- use of a cell line or non-human animal according to the invention in screening for an agent for use in diagnosis, prevention or treatment of an individual having a genetic predisposition to inflammatory bowel disease;
- an apparatus arranged to perform a method of diagnosis according to the invention

comprising:

(i) means for receiving genetic information;

(ii) a module for comparing the genetic information with a database comprising information relating to a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease; and

(iii) means for determining on the basis of said comparison the susceptibility of the individual to inflammatory bowel disease;

- a computer program comprising program code means that, when executed on a computer system, instruct the computer system to perform a method of diagnosis according to the invention;
- a computer program product comprising a computer-readable storage medium having recorded thereon a computer program according to the invention;
- a database comprising information relating to a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease;
- a computer program product comprising program code means on a carrier wave, which program code means, when executed on a computer system, instruct the computer system to perform a method of diagnosis according to the invention; and
- a method for determining whether or not an individual is predisposed to inflammatory bowel disease, which method comprises:

(i) inputting genetic data from said individual to a computer system;

(ii) comparing said genetic data to a computer database, which database comprises information relating to a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease; and

(iii) determining on the basis of said comparison whether said individual has said polymorphism thereby determining whether the individual is predisposed to inflammatory bowel disease.

Description of the sequences mentioned herein

SEQ ID NO: 1 shows the CCRL2 cDNA.

SEQ ID NO: 2 shows the sequence of the CCRL2 protein.

Brief Description of the Drawings

Figure 1 shows sequence chromatogram for a homozygous sample (top two traces) and a heterozygous sample.

Figure 2 is a CCRL2 gene illustration showing the positions of the polymorphisms shown in Table 4.

Figure 3 shows Taqman Results Tissue type versus Normalised Quantity.

Figure 4 shows Taqman results for Cell lines versus Normalised Quantity

Figure 5 shows Affymetrix Results Tissue versus Signal. Bars are shaded to represent detection call (Present, white; marginal, grey; absent, black). The two probe sets are grouped together for each tissue (TPC2138_s_at then TPC2078s_at).

Detailed Description of the Invention

The invention provides diagnosis and therapy of inflammatory bowel disease Preferably the inflammatory bowel disease is ulcerative colitis. Inflammatory bowe disease may be present, or be suspected of being present, in the individual to be diagnose or treated. The individual is typically a mammalian individual, for example a mammal kep as a pet or for agricultural or sporting reasons. In one embodiment the mammal is one i which inflammatory bowel disease occurs naturally (without intervention by man). Th mammal may be a bovine, porcine, canine, feline, rodent (such as a mouse, rat or hamster) or primate animal. In a preferred embodiment the individual is a human individual.

Diagnosis

The present invention provides a method of determining whether an individual is predisposed to inflammatory bowel disease, which method comprises identifying whether the individual has a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease. CCRL2 cDNA is shown in SEQ ID NO: 1 and the translated peptide sequence is shown in SEQ ID NO: 2. The "CCRL2 protein" is the protein encoded by a CCRL2 polynucleotide and includes any naturally occurring isoform of this protein (in diagnosis the isoform of CCRL2 which is typed will of course be the isoform which is present in the individual who is being tested). Such an isoform will generally comprise all or part of the sequence of SEQ ID NO: 2, or comprise sequence which has sequence has homology with (all or part of) SEQ ID NO: 2. Preferably the isoform has sequence identity to SEQ ID NO: 2 except at the polymorphic position(s).

The polymorphism which is detected may be one or more of those as set out in Table 4, in particular the SNP CCRL2-204 or CCRL-202, or a polymorphism which is linkage disequilibrium with any of these polymorphisms.

Polymorphisms which are in linkage disequilibrium with each other in a population are found together on the same chromosome. Typically one is found at least 30% of the times, for example at least 40 %, 50%, 70% or 90%, of the time the other is found on a particular chromosome in individuals in the population. Thus polymorphisms that are not functional susceptibility polymorphisms, but are in linkage disequilibrium with the functional polymorphisms, may act as a marker indicating the presence of the functional polymorphism. Polymorphisms which are in linkage disequilibrium with any of the polymorphisms mentioned herein are typically within 500kb, preferably within 400kb, 200kb, 100 kb, 50kb, 10kb, 5kb or 1 kb of that polymorphism.

The method according to the first aspect of the invention may comprise contacting a sample from an individual with a specific binding agent for the polymorphism and determining whether the agent binds to the polymorphism, wherein binding of the agent to the polymorphism indicates that the individual is predisposed to inflammatory bowel disease. The polymorphism which is being detected is a polymorphism in the CCRL2 polynucleotide or protein.

In the method a genetic predisposition is diagnosed.

The method may be carried out *in vivo*, however typically it is carried out *in vitro* on a sample from the individual. The sample typically comprises a body fluid and/or cells of the individual and may, for example, be obtained using a swab, such as a mouth swab. The sample may be a blood, urine, saliva, skin, cheek cell or hair root sample.

The sample is typically processed before the method is carried out, for example DNA extraction may be carried out. The polynucleotide or protein in the sample may be cleaved either physically or chemically (e.g. using a suitable enzyme). In one embodiment the part of polynucleotide in the sample is copied (or amplified), e.g. by cloning or using a PCR based method prior to detecting the polymorphism(s).

The polymorphism may be one or more of the polymorphisms as set out in Table 4. This table includes a number of "silent" polymorphisms. Such "silent" polymorphisms are those which do not result in a change in amino acid. Such polymorphisms may or may not be causative. However, a non-causative polymorphism nevertheless provides an association to an individual being predisposed to inflammatory bowel disease by being in linkage disequilibrium with the causative SNP. The polymorphism may also affect the function of

the protein in another way, for example by altering gene expression by changing promoter activity, mRNA stability or mRNA splicing.

In a method according to the first aspect of the invention, the polymorphism in the CCRL2 polynucleotide may be determined for one or both alleles of the individual.

In the present invention, any one or more methods may comprise determining the presence or absence of one or more polymorphisms in the individual. This applies to all aspects of the invention. Where two polymorphisms are involved, the first is in accordance with the present invention and the second (or further) may or may not be in accordance with the present invention. Preferably, when two or more polymorphisms are involved, at least one is for the presence or absence of the SNP CCRL2-204 or CCRL2-202 as set out in Table 4. The second or further polymorphism may also be set out in Table 4.

Thus in one embodiment of the method the presence or absence of more than one of the specific polymorphisms in Table 4 is detected, such as at least 2, 3, 5 or 8 of these polymorphisms, i.e. any possible combination of these polymorphism may be tested. Preferred combinations of polymorphisms include those in which the presence/absence of the CCRL-204 and CCRL-202 polymorphisms is detected.

The invention also provides a method of identifying a polymorphism which can be used to determine whether an individual has a genetic predisposition to inflammatory bowe disease, the method comprising screening the CCRL2 polynucleotide or polypeptide in one or more individuals.

Detection of polymorphisms

The polymorphism is typically detected by directly determining the presence of the polymorphism sequence in a polynucleotide or protein of the individual. Such a polynucleotide is typically genomic DNA, mRNA or cDNA. The polymorphism may be detected by any suitable method such as those mentioned below.

A specific binding agent is an agent that binds with preferential or high affinity to the protein or polypeptide having the polymorphism but does not bind or binds with only low affinity to other polypeptides or proteins (such as a CCRL2 protein or polynucleotide which does not comprise the polymorphism).

The specific binding agent may be a probe or primer. The probe may be a protein (such as an antibody) or an oligonucleotide. The probes or primers will typically also bind to flanking nucleotides and amino acids on one or both sides of the polymorphism, for example at least 2, 5, 10, 15 or more flanking nucleotide or amino acids in total or on each

side. Thus a probe or primer may be fully or partially complementary to either all or part of the flanking 5' and/or 3' sequences shown in the Table 4. The probe may be labelled or may be capable of being labelled indirectly. The binding of the probe to the polynucleotide or protein may be used to immobilise either the probe or the polynucleotide or protein.

Generally in the method, determination of the binding of the agent to the polymorphism can be done by determining the binding of the agent to the polynucleotide or protein of the individual. However in one embodiment the agent is also able to bind the corresponding wild-type sequence, for example by binding the nucleotides or amino acids which flank the polymorphism position, although the manner of binding to the wild-type sequence will be detectably different to the binding of a polynucleotide or protein containing the polymorphism.

The method may be based on an oligonucleotide ligation assay in which two oligonucleotide probes are used. These probes bind to adjacent areas on the polynucleotide which contains the polymorphism, allowing (after binding) the two probes to be ligated together by an appropriate ligase enzyme. However the presence of single mismatch within one of the probes may disrupt binding and ligation. Thus ligated probes will only occur with a polynucleotide that contains the polymorphism, and therefore the detection of the ligated product may be used to determine the presence of the polymorphism.

In one embodiment the probe is used in a heteroduplex analysis based system. In such a system when the probe is bound to polynucleotide sequence containing the polymorphism it forms a heteroduplex at the site where the polymorphism occurs (i.e. it does not form a double strand structure). Such a heteroduplex structure can be detected by the use of single or double strand specific enzyme. Typically the probe is an RNA probe, the heteroduplex region is cleaved using RNAase H and the polymorphism is detected by detecting the cleavage products.

The method may be based on fluorescent chemical cleavage mismatch analysis which is described for example in PCR Methods and Applications 3, 268-71 (1994) and Proc. Natl. Acad. Sci. 85, 4397-4401 (1998).

In one embodiment a PCR primer is used that primes a PCR reaction only if it binds a polynucleotide containing the polymorphism (i.e. a sequence- or allele-specific PCR system) and the presence of the polymorphism may be determined by the detecting the PCR product. Preferably the region of the primer which is complementary to the polymorphism is at or near the 3' end of the primer. The presence of the polymorphism may be determined

using a fluorescent dye and quenching agent-based PCR assay such as the Taqman PCR detection system.

The specific binding agent may be capable of specifically binding the amino acid sequence encoded by a polymorphic sequence, preferably one of the sequences shown in Table 4. For example, the agent may be an antibody or antibody fragment. The detection method may be based on an ELISA system.

The method may be an RFLP based system. This can be used if the presence of the polymorphism in the polynucleotide creates or destroys a restriction site that is recognised by a restriction enzyme.

The presence of the polymorphism may be determined based on the change which the presence of the polymorphism makes to the mobility of the polynucleotide or protein during gel electrophoresis. In the case of a polynucleotide single-stranded conformation polymorphism (SSCP) or denaturing gradient gel electrophoresis (DDGE) analysis may be used.

In another method of detecting the polymorphism a polynucleotide comprising the polymorphic region is sequenced across the region which contains the polymorphism to determine the presence of the polymorphism.

Bioinformatics

The sequences of the polymorphisms in the CCRL2 gene associated with inflammatory bowel disease may be stored in an electronic format, for example in a computer database. The database may include further information about the polymorphism. For example the database may provide one or more aspects of the following types of information: the level of association of the polymorphism with inflammatory bowel disease, the frequency of the polymorphism in inflammatory bowel disease patients, the probability of a patient having that polymorphism developing inflammatory bowel disease, the interaction of a polynucleotide or polypeptide having the polymorphism with a therapeutic agent. Accordingly, a database comprising information relating to a polymorphism in the CCRL2 gene associated with inflammatory bowel disease is provided by the invention. A database of the invention may include additional genetic information such as polymorphisms in other genes which are associated with inflammatory bowel disease.

A method of determining whether or not an individual is predisposed to inflammatory bowel disease according to the invention may be carried out by electronic means, for example using a computer system. Accordingly, the present invention provides

a method for determining whether or not an individual is predisposed to inflammatory bowel disease, which method comprises:

(i) inputting genetic data from said individual to a computer;

(ii) comparing said genetic data to a computer database, which database comprises information relating to a polymorphism in the CCRL2 gene which polymorphism is associated with inflammatory bowel disease; and

(iii) determining on the basis of said comparison whether said individual has said polymorphism, thereby determining whether the individual is predisposed to inflammatory bowel disease.

The invention also provides an apparatus arranged to perform a method according to the invention, which method comprises:

(i) an apparatus arranged to perform a method of diagnosis according to the invention comprising:

(i) means for receiving genetic information;

(ii) a module for comparing the genetic information with a database comprising information relating to a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease; and

(iii) means for determining on the basis of said comparison the susceptibility of the individual to inflammatory bowel disease.

Preferably the database comprises information relating to two or more polymorphisms which are associated with inflammatory bowel disease including polymorphisms in the CCRL2 gene and polymorphisms in genes other than the CCRL2 gene, which polymorphisms are also associated with inflammatory bowel disease. The apparatus may comprise means for determining the susceptibility of the individual to inflammatory bowel disease based on the combination of polymorphisms present in the genetic information of said individual.

The invention also provides a computer program comprising program code means that, when executed on a computer system, instruct the computer system to perform a method of diagnosis according to the invention. A computer program product comprising a computer-readable storage medium having recorded thereon a computer program of the invention is also provided. A computer program product comprising program code means on a carrier wave that, when executed on a computer system, instruct the computer system to perform a method of the invention is additionally provided.

A computer system may also be used to determine the suitability of an individual to

treatment using a particular therapeutic agent. A computer system suitable for such a use, typically comprises means for determining whether an individual predisposed to inflammatory bowel disease has a polymorphism that enhances or reduces the efficacy of an agent used in the treatment of inflammatory bowel disease.

Treatment of diagnosed patients

The invention provides a method for treating an individual who has been identified as having a predisposition to inflammatory bowel disease by a method of the invention, comprising administering to the individual an effective amount of an agent which prevents or treats inflammatory bowel disease; or an agent which prevents or treats a symptom of inflammatory bowel disease. The substance may be administered to a patient to prevent the onset of inflammatory bowel disease.

The invention also provides:

- use of an agent in the manufacture of a medicament for use in treating a patient who has been diagnosed as being susceptible to inflammatory bowel disease by a method of the invention; and
- a pharmaceutical pack comprising the agent and instructions for administering the agent to humans identified by the method of the invention.

Typically the following substances may be used to prevent or treat to inflammatory bowel disease or a symptom of to inflammatory bowel disease: aminosalicylates, corticosteroids, anti-TNFs (such as antibodies against TNF, e.g. infliximab), antibiotics and probiotics.

Screening for therapeutic agents

The invention provides a method for identifying an agent for the prevention or treatment of inflammatory bowel disease, the method comprising:

(i) contacting a test agent with a CCRL2 polypeptide or polynucleotide having a mutation or polymorphism associated with inflammatory bowel disease; and

(ii) determining whether the test agent is capable of binding to the polypeptide or polynucleotide and modulating the activity or expression of the polypeptide or polynucleotide.

The method may be carried out *in vitro* (inside or outside a cell) or *in vivo*. In one embodiment the method is carried out on a cell, cell culture or cell extract which comprises the CCRL2 polypeptide or polynucleotide. The cell may be any of the cells mentioned

herein, and is preferably the cell is one in which the product is naturally expressed, such as an endothelial or colon cell.

The method may be carried out in an animal that is a knockout for CCRL2 or that comprises a CCRL2 gene comprising a polymorphism which causes susceptibility to inflammatory bowel disease, such as any such polymorphism mentioned herein (such as any suitable animal mentioned herein, including the animals which are transgenic for CCRL2 polymorphisms or knockouts for CCRL2 which are mentioned below).

Any suitable binding assay format can be used to determine whether the product binds the test agent, such as the formats discussed below.

The term 'modulate' includes any of the ways mentioned herein in which the agent of the invention is able to modulate CCRL2. This includes upregulation or downregulation of CCRL2 expression, upregulation or downregulation of CCRL2 degradation, stimulation or inhibition of CCRL2 receptor activity, including potentiation of CCRL2 activity in response to an endogenous chemokine. The ability of a test agent to modulate the activity or expression of CCRL2 may be determined by contacting CCRL2 with the test agent unde conditions that permit activity or expression of CCRL2 and comparing CCRL2 activity in the presence and absence of the test agent. Preferably, the modulation is a correction of aberrant CCRL2 activity or expression (such as aberrant activity or expression caused by any of the types of polymorphism or specific polymorphisms mentioned herein).

The assay may measure the effect of the test agent on the binding between CCRL2 and another substance, preferably a chemokine. Suitable assays in order to measure the changes in such interactions include fluorescence imaging plate reader assays, and radioligand binding assays. The assay may measure the effect of the test agent on CCRL2 activity. Preferably such assays involve monitoring activation of a G-protein coupled response. To identify an antagonist, an agonist of CCRL2 activity may be included in the activity assay.

Suitable test agents which tested in the above screening methods include antibody agents (for example, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies and CDR-grafted antibodies) or aptamer agents. The antibody agent may have binding affinity for the CCRL2 receptor or for an endogenous chemokine. Furthermore, combinatorial libraries, defined chemical identities, peptide and peptide mimetics, oligonucleotides and natural agent libraries, such as display libraries (e.g. phage display libraries) may also be tested. Oligonucleotide libraries, such as aptamer libraries may be tested.

The test agents may be chemical compounds, which are typically derived from synthesis around small molecules which may have any of the properties of the agent mentioned herein (such as the organic compounds mentioned herein). Batches of the test agents may be used in an initial screen of, for example, ten agents per reaction, and the agents of batches which show modulation tested individually.

Where CCRL2 activity or expression is reduced in a subject having inflammatory bowel disease, an agent for use in the treatment or prevention of inflammatory bowel disease is preferably an agonist or potentiator of CCRL2 activity or an agent which enhances expression of CCRL2. Where CCRL2 activity or expression is enhanced in a subject having inflammatory bowel disease, a therapeutic agent is typically an antagonist of CCRL2 activity or an inhibitor of expression. The agent may bind to an endogenous chemokine that interacts with the CCRL2 receptor to prevent receptor activation, for example the agent may be an antibody to an endogenous chemokine.

The invention further provides an agent identified by such a method and the use of such an agent in the manufacture of a medicament for use in preventing or treating inflammatory bowel disease. A pharmaceutical composition for the prevention or treatmen of inflammatory bowel disease comprising an agent of the invention and a pharmaceutically acceptable carrier or diluent is also provided.

Accordingly, the invention also provides a method of formulating a pharmaceutical composition comprising:

(i) identifying an agent for the prevention or treatment of inflammatory bowel disease by a method according to the invention; and

(ii) formulating the agent with a pharmaceutically acceptable carrier or diluent.

A method of preventing or treating inflammatory bowel disease is provided by the invention, which method comprises administering a therapeutically effective amount of an agent according to the invention to an individual in need thereof.

A method of treating inflammatory bowel disease of the invention typically comprises:

(i) identifying an agent for the prevention or treatment of inflammatory bowel disease by a method according to the invention; and

(ii) administering a therapeutically effective amount of an agent identified in (i) to an individual having inflammatory bowel disease.

Polypeptides and polynucleotides

The invention provides a CCRL2 protein which contains a polymorphism that causes, contributes or predisposes to or is associated with inflammatory bowel disease. SEQ ID NO: 2 shows the sequence of a CCRL2 protein. A CCRL2 protein of the invention has a sequence that is variant of SEQ ID NO: 2 at the polymorphic position(s). Such a protein is preferably one encoded by a polynucleotide having the CCRL201, CCRL202, CCRL203, CCRL204 or CCRL205 polymorphism shown in Table 4, more preferably the CCRL202 or CCRL204 polymorphism.

The invention also provides a polynucleotide which comprises a polymorphism which causes/contributes to or is associated with inflammatory bowel disease. The polymorphism may be in linkage disequilibrium with the functional polymorphism which causes/contributes to inflammatory bowel disease. Such a polynucleotide is preferably a polynucleotide comprising a sequence which is identical to the sequence shown in SEQ ID NO: 1 except for nucleic acid insertion(s), deletion(s) or substitution(s) at one or more polymorphic positions. Preferably the polymorphism is one shown in Table 4. More preferably the polymorphism is CCRL204 or CCRL202.

The polynucleotide is typically at least 10, 15, 20, 30, 50, 100, 200, 500, bases long, such as at least (or up to) 1kb, 10kb, 100kb, 1000 kb or more in length. The polynucleotide may be RNA or DNA, including genomic DNA, synthetic DNA or cDNA. The polynucleotide may be single or double stranded.

The polynucleotide may comprise synthetic or modified nucleotides, such as methylphosphonate and phosphorothioate backbones or the addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule.

A polynucleotide of the invention may be used as a primer (e.g. for PCR) or a probe. A polynucleotide or polypeptide of the invention may carry a revealing label. Suitable labels include radioisotopes such as ^{32}P or ^{35}S, fluorescent labels, enzyme labels or other protein labels such as biotin.

The invention also provides expression vectors that comprise polynucleotides of the invention and are capable of expressing a polypeptide of the invention. Such vectors may also comprise appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Thus the coding sequence in

the vector is operably linked to such elements so that they provide for expression of the coding sequence (typically in a cell). The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner.

The vector may be for example, plasmid, virus or phage vector. Typically the vector has an origin of replication. The vector may comprise one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example for the production of DNA or RNA or used to transfect or transform a host cell, for example, a mammalian host cell. The vectors may also be adapted to be used *in vivo*, for example in a method of gene therapy.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe nmt*1 and *adh* promoter. Mammalian promoters include the metallothionein promoter which can be induced in response to heavy metals such as cadmium. Viral promoters such as the SV40 large T antigen promoter or adenovirus promoters may also be used.

Mammalian promoters, such as β-actin promoters, may be used. Tissue-specific promoters are especially preferred. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR), the rous sarcoma virus (RSV) LTR promoter, the SV40 promoter, the human cytomegalovirus (CMV) IE promoter, adenovirus, HSV promoters (such as the HSV IE promoters), or HPV promoters, particularly the HPV upstream regulatory region (URR).

The vector may further include sequences flanking the polynucleotide giving rise to polynucleotides which comprise sequences homologous to eukaryotic genomic sequences, preferably mammalian genomic sequences, or viral genomic sequences. This will allow the introduction of the polynucleotides of the invention into the genome of eukaryotic cells or viruses by homologous recombination. In particular, a plasmid vector comprising the expression cassette flanked by viral sequences can be used to prepare a viral vector suitable for delivering the polynucleotides of the invention to a mammalian cell. Other examples of suitable viral vectors include herpes simplex viral vectors and retroviruses, including lentiviruses, adenoviruses, adeno-associated viruses and HPV viruses. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide giving rise to the polynucleotide

into the host genome. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression.

The polynucleotide may be a probe or primer which is capable of selectively binding to a CCRL2 nucleic acid or protein. Preferably the probe or primer is capable of selectively binding to a polymorphism in the nucleic acid or protein. The probe or primer more preferably comprises a nucleic acid sequence corresponding to a SNP in the CCRL2 gene associated with an individual's predisposition to inflammatory bowel disease (such as in Table 4). The invention thus provides a probe or primer for use in a diagnostic method according to the invention, which probe or primer is capable of selectively detecting a polymorphism in the CCRL2 polynucleotide associated with inflammatory bowel disease.

Preferably the probe is isolated or recombinant nucleic acid. Preferably it is from 10, 15, 20, 25 bases in length. It may correspond to or be antisense to the sequences set out in Table 4.

The probe may be immobilised on an array, such as a "DNA chip".

It is understood that any of the above features that relate to polynucleotides and proteins may also be a feature of the other polypeptides and proteins mentioned herein, sucl as the polypeptides and proteins used in the screening and therapeutic aspects of the invention. In particular such features may be any of the lengths, modifications and vectors forms mentioned above.

Treatment using polypeptides and polynucleotides

The invention provides the use of a CCRL2 polynucleotide or polypeptide, not having a mutation or polymorphism associated with inflammatory bowel disease, in the manufacture of a medicament for the prevention or treatment of inflammatory bowel disease.

In particular, the invention provides a method for treating or preventing inflammatory bowel disease in an individual comprising:

(i) identifying, in the individual, the presence of a polymorphism in the CCRL2 polynucleotide or CCRL2 protein which is associated with inflammatory bowel disease; and

(ii) introducing into the individual a CCRL2 polynucleotide of the invention which is a different allele of the polymorphism or polymorphisms to that present in the individual.

Preferably, the CCRL2 polynucleotide introduced into the individual

comprises the sequence shown in SEQ ID NO:1.

Thus the invention includes use of a polypeptide with CCRL2 activity, or a polynucleotide capable of expressing such a polypeptide, in the manufacture of a medicament for the prevention or treatment of inflammatory bowel disease. The polypeptide or polynucleotide may be in cellular form, such as in the form of any of the cells mentioned herein. The polypeptide or polynucleotide may be in any of the forms or have any of the features mentioned herein in regard to polypeptides or polynucleotides.

The polypeptide with CCRL2 activity is typically

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 2

(b) a homologue of (a), or

(c) a fragment of (a) or (b) which has a length of at least 15 amino acids.

In one embodiment the polypeptide has at least 80% identity to the amino acid sequence of SEQ ID NO: 2, a fragment thereof which has a length of at least 15 amino acid or is a fusion protein which (i) comprises sequence which has at least 80% identity to the amino acid sequence of SEQ ID NO: 2, or (ii) comprises the said fragment.

The polynucleotide which is capable of expressing the polypeptide generally comprises a sequence:

(a) which is the same SEQ ID NO: 1, or complementary thereto;

(b) which hybridises to (a);

(c) that is degenerate as a result of the genetic code to a sequence as defined in (a) or (b); or

(d) having at least 80% identity to a sequence as defined in (a), (b) or (c).

Such hybridisation may be performed under conditions of medium to high stringency (for example 0.03M sodium chloride and 0.003M sodium citrate at from about 50°C to about 60°C).

Cell Lines and Transgenic animals

The invention provides a cell line comprising a CCRL2 polynucleotide having a polymorphism associated with inflammatory bowel disease. The cell line may have been modified to express the polypeptide of the invention. Such cell lines include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, lower eukaryotic cells, such as yeast, or prokaryotic cells such as bacterial cells. Particular examples of cells which may be modified by insertion of vectors encoding for a polypeptide according to the invention include mammalian HEK293T, CHO, HeLa and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for

mature glycosylation of a polypeptide. Expression may be achieved in transformed oocytes.

A non-human animal which is transgenic for a CCRL2 polynucleotide having a polymorphism associated with inflammatory bowel disease or is a knockout for CCRL2 is also provided by the invention. Such an animal may thus not be able to express a functional CCRL2 or may express a CCRL2 protein with impaired/decreased or aberrant activity. In one embodiment a polynucleotide having a polymorphism associated with inflammatory bowel disease has been introduced into the genome of the animal, and so the animal may have such an introduced polynucleotide in addition to its original CCRL2 alleles (which original alleles may or may not comprise a polymorphism associated with inflammatory bowel disease.

The animal may be any suitable mammal such as a rodent (e.g. a mouse, rat or hamster) or primate. Typically the genome of all or some of the cells of the animal comprises a polynucleotide of the invention. Generally the animal expresses a protein of the invention. Typically the animal suffers from inflammatory bowel disease and can, therefore, be used in a method to assess the efficacy of agents in relieving inflammatory bowel disease.

Thus the invention provides the use of a cell line or a non-human animal according to the invention in screening for an agent for use in diagnosis, prevention or treatment of an individual having a genetic predisposition to inflammatory bowel disease.

Testing efficacy

The effectiveness of particular agents which prevent or treat inflammatory bowel disease may be affected by or dependent on whether the individual has particular polymorphisms in the to CCRL2 gene region or protein. Accordingly, a still further aspect of the invention provides a method for determining the efficacy of an agent useful in the treatment of inflammatory bowel disease in an individual having a polymorphism associated with inflammatory bowel disease in the CCRL2 polynucleotide, which method comprises:

(i) providing a CCRL2 polynucleotide having said polymorphism, or a protein encoded thereby;

(ii) contacting said protein or gene with said agent; and

(iii) determining whether said protein or gene interacts with said agent.

The gene or protein may be in a subject having the polymorphism or in a non-human

animal according to the invention. Step (iii) may comprise monitoring the effect of administering the agent on one or more phenotype of inflammatory bowel disease.

The invention also includes a method of treating a patient who has been identified as being able to respond to the agent comprising administering the agent to the patient.

Similarly certain agents may produce side effects in individuals with particular polymorphisms in the CCRL2 gene region or protein. Thus the invention can also allow the identification of a patient who is at increased risk of suffering side effects due to such an agent by identifying whether an individual has such a polymorphism.

In a further aspect, the invention may further be used in the development of new drug therapies which selectively target one or more allelic variants of the CCRL2 protein (i.e. which have different polymorphisms). Identification of a link between a particular allelic variant and predisposition to condition development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate th biological activity of the variants implicated in the condition process while minimising effects on other variants.

Homologues

Homologues of polynucleotide or protein sequences are referred to herein. It also t be understood that where appropriate in any of the embodiments mentioned herein that relate to CCRL2 polynucleotides or proteins/polypeptides, homologues and/or fragments of SEQ ID NO's 1 and 2 may be used instead.

Such homologues typically have at least 70% homology, preferably at least 80, 90%, 95%, 97% or 99% homology, for example over a region of at least 15, 20, 30, 100 more contiguous nucleotides or amino acids. In many of the embodiments mentioned herein the polynucleotide or proteins will be homologues of SEQ ID NO's 1 and 2.

The homology may be calculated on the basis of nucleotide or amino acid identity (sometimes referred to as "hard homology").

For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* 12, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (such as identifying equivalent or corresponding sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The homologous sequence typically differ by at least 1, 2, 5, 10, 20 or more mutations (which may be substitutions, deletions or insertions of nucleotide or amino acids). These mutations may be measured across any of the regions mentioned above in relation to calculating homology. In the case of proteins the substitutions are preferably conservative substitutions. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

Antibodies

The invention also provides antibodies specific for a polypeptide of the invention. The antibodies include those which are specific for CCRL2 proteins which have a polymorphism, such as any of the polymorphisms mentioned herein, but which do not bind the corresponding wild-type CCRL2 protein. Thus particular antibodies of the invention do not bind the sequence shown in SEQ ID NO: 2 or fragments of that sequence, but do bind to CCRL2 proteins that have polymorphisms which cause a disease condition mentioned herein or polymorphisms which are in linkage disequilibrium therewith. Preferred antibodies specifically bind to a protein encoded by a polynucleotide having the CCRL201, CCRL202, CCRL203, CCRL204 or CCRL205 polymorphism shown in Table 4, more preferably the CCRL202 or CCRL204 polymorphism.

The antibodies of the invention are for example useful in purification, isolation or screening methods involving immunoprecipitation techniques or, indeed, as therapeutic agents in their own right.

Accordingly, the present invention provides a method of treating or preventing inflammatory bowel disease, which method comprises administering a therapeutically effective amount of an antibody of the invention to an individual in need thereof.

Antibodies may be raised against specific epitopes of the polypeptides of the invention. An antibody, or other compound, "specifically binds" to a polypeptide when it binds with preferential or high affinity to the protein for which it is specific but does substantially bind not bind or binds with only low affinity to other polypeptides. A variety of protocols for competitive binding or immunoradiometric assays to determine the specific binding capability of an antibody are well known in the art (see for example Maddox *et al*, J. Exp. Med. 158, 1211-1226, 1993). Such immunoassays typically involve the formation

of complexes between the specific protein and its antibody and the measurement of complex formation.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments which bind a polypeptide of the invention. Such fragments include Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragment thereof may be chimeric antibodies, CDR-grafted antibodies or humanised antibodies.

Antibodies may be used in a method for detecting polypeptides of the invention in a biological sample (such as any such sample mentioned herein), which method comprises:

I providing an antibody of the invention;

II incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and

III determining whether antibody-antigen complex comprising said antibody is formed.

Antibodies of the invention can be produced by any suitable method. Means for preparing and characterising antibodies are well known in the art, see for example Harlow and Lane (1988) "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. For example, an antibody may be produced by raising antibody in a host animal against the whole polypeptide or a fragment thereof, for example an antigenic epitope thereof, herein after the "immunogen". The fragment may be any of the fragments mentioned herein (typically at least 10 or at least 15 amino acids long) and comprise a polymorphism (such as any of the polymorphisms mentioned herein).

A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the animal's serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified.

A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* **256**, 495-497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation

of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat, mouse, guinea pig, chicken, sheep or horse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

Diagnostic kit

The invention also provides a diagnostic kit that comprises a specific binding agent, probe, primer (or pair/combination of primers) or antibody (including an antibody fragment) as defined herein (capable of detecting or aiding detection of a polymorphism).

The primer or pair of primers may be sequence specific primers which only caus PCR amplification of a polynucleotide sequence comprising the polymorphism to b detected (as discussed herein).

The kit may also comprise a specific binding agent, probe, primer (o pair/combination of primers) or antibody which is capable of detecting the wild-typ sequence of the polymorphism.

The kit may additionally comprise one or more other reagents or instruments which enable any of the embodiments of the method mentioned above to be carried out. Such reagents or instruments include one or more of the following: a means to detect the binding of the agent to the polymorphism, a detectable label (such as a fluorescent label), an enzyme able to act on a polynucleotide (typically a polymerase, restriction enzyme, ligase, RNAse H or an enzyme which can attach a label to a polynucleotide), suitable buffer(s) (aqueous solutions) for enzyme reagents, PCR primers which bind to regions flanking the polymorphism (e.g. the primers discussed herein), a positive and/or negative control, a gel electrophoresis apparatus, a means to isolate DNA from sample, a means to obtain a sample from the individual (such as swab or an instrument comprising a needle) or a support comprising wells on which detection reactions can be done.

The kit may be, or include, an array such as a "DNAchip" comprising the specific binding agent, preferably a probe, of the invention.

Administration

The formulation of any of the therapeutic agents mentioned herein, including polypeptides, polynucleotides and antibodies, will depend upon factors such as the nature of the agent and the condition to be treated. Any such agent may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), parenterally, subcutaneously, intravenously, intramuscularly, intrasternally, transdermally or by infusion techniques. The agent may also be administered as suppositories. A physician will be able to determine the required route of administration for each particular patient.

Typically the agent is formulated for use with a pharmaceutically acceptable carrier or diluent. The pharmaceutical carrier or diluent may be, for example, an isotonic solution. For example, solid oral forms may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film coating processes.

Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for intravenous or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

A therapeutically effective amount of agent is administered. A therapeutically effective amount of an agent is an amount that alleviates the symptoms of inflammatory

bowel disease or which prevents or delays the onset of symptoms of inflammatory bowel disease.

The dose may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg, preferably from about 0.1mg/kg to 10mg/kg of body weight, according to the activity of the specific inhibitor, the age, weight and conditions of the subject to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g.

The present invention is described with reference to the following, non-limiting Examples:

Example 1: Determination of CCRL2 Gene Structure, Fragment design and PCR amplification

Exons were aligned to the 1.6Kb cDNA transcript (NM_003965) using the Retrieve Exons program. Retrieve Exons is a proprietary software pipeline which aligns a cDNA sequence with the corresponding genomic DNA sequence, identifies exon boundaries within the cDNA sequence and masks repetitive DNA sequences within the genomic sequence. The software also identifies the sequences of dbSNPs located within and around the gene and exports the cDNA, genomic DNA , masked genomic DNA, exon and dbSNP sequences into an accessible flat file format that can be imported into Sequencher sequence analysis program. Repetitive regions were avoided in positioning oligonucleotides for fragment design. Intronic oligonucleotides were designed to flank the exons. The oligonucleotide sequences for amplification of the CCRL2 gene are presented in Table 1.

Example 2: Detection of Mutant Alleles

To identify novel polymorphisms in the human CCRL2 gene, exons encoding the 1.6kb transcript were analysed by Denaturing High Performance Liquid Chromatography (DHPLC) using a Transgenomic WAVE (Transgenomic Inc). This method is based on an ion-pair reverse-phase chromatography, which separates DNA according to the melting properties of the sequence. The DNA is then eluted on an acetonitrile gradient from the column and viewed as a chromatogram peak. At a specific pre-determined temperature,

DNA homoduplexes and heteroduplexes are resolved and can therefore be distinguished from each other.

To determine the optimum temperature for mutation detection, melting curves were empirically calculated by running each fragment at series of temperatures. Melting domains were analysed for each fragment and the optimum temperature identified by selecting the temperature at which the DNA fragment is 25% melted. This was carried out in conjunction with the WAVEMAKER Software (Transgenomic Inc), which predicts the temperature at which the double stranded DNA fragment will melt into separate strands. The mutation detection temperatures selected for each fragment are detailed in Table 1.

DNA samples from 23 control individuals were amplified by PCR using the oligonucleotides and conditions in Tables 1-3. Individuals were analysed separately by DHPLC to scan for heteroduplex samples indicative of the presence of polymorphisms in the DNA fragment. Heteroduplexes were identified based on separation differences when compared to homozygous individuals.

To identify the position and nature of the polymorphism, PCR products were sequenced initially using the Dynamic ET Terminator on an ABI 377 sequencer.

Dynamic ET Terminator cycle sequencing was conducted following the sequencing kit protocol (Amersham, Cat. No. US80890). Each reaction consisted of 200ng template DNA, 0.25 pmol primer, 8µl Termination mix and distilled water to 20µl. The cycling conditions used were; 96°C for 30 sec, 50°C for 20 sec, 60°C for 1 min, cycled 25 times, then hold at 4°C until ready to purify. Sequencing products were precipitated by ethanol and Sodium Acetate (NaAc). Dry pellets were resuspended in 2µl deionised formamide and denatured at 95°C for 3 mins prior to loading on a 48cm sequencing gel and running for 10 hours on an ABI automatic 377 sequencer.

The sequence chromatogram files were imported into the Sequencher sequence analysis program (GENE CODES Corp) and aligned to search for sequence variations. Products from both heterozygous and wild type individuals were sequenced in forward and reverse orientations to compare traces (Figure 1).

Table 1: Annealing temperatures, primer sequences and TG Wave mutation and detection temperatures used for each CCRL2 scanning fragment.

Oligo Name	Sequence 5' —› 3'	Ta °C	Mutation detection temperatures.
CCRL2 Pr1v2 F	CACACCGTGACTGTCATGGC	68	58/63
CCRL2 Pr1v2 R	CACAGATGACTTACCACTGGGC		
CCRL2 Pr2v2 F	GTCTGATCAAAAGGAGGGCATCC	68	59
CCRL2 Pr2v2 R	CTGGACCTGGATTCATACAGCC		
CCRL2x1.1F	GTCTTCTGAAATAGGGAATTACTCTGGC	61	60
CCRL2x1.1 R	CAGGCAAAGTGGCCAGAATGGC		
CCRL2x1.2 F	CCTTCTGACTGTGCAAAGGTACC	61	59
CCRL2x1.2 R	GTCCAGATTGTAGCTGCTCTTGC		
CCRL2x1.3 F	GTTCAGGGAGCAGAGGTATAGC	61	53/61
CCRL2x1.3 R	CCTCTCCCTTTTCTTCCTGTATCC		

Table 2: PCR amplification conditions for the CCRL2 fragments.

REAGENT	VOLUME
DNA template (50ng)	5µl
Forward primer (0.2µM)	1µl
Reverse primer (0.2µM)	1µl
10X Reaction Buffer	5µl
25mM $MgCl_2$ (2.5mM)	5µl
2.5mM dNTPs (250µM)	5µl
Amplitaq Gold (2.5U/µl)	0.5µl
H_2O	27.5µl
TOTAL	50µl

Table 3: PCR cycling conditions for amplification of the CCRL2 fragments

Initial denaturation	12 min at 95°C	
Denaturation	30 sec at 95°C	35 cycles
Annealing	30 sec at Ta°C	
Extension	30 sec at 72°C	
Final extension	5 min at 72°C	

Table 4: Details of each SNP found by DHPLC on the TG Wave and sequencing.

SNP Name	5' Flanking sequence	IUB code	3'Flanking sequence	Functional Effect
CCRL201	AAATATAAAGGACTCAA ACGCGTGGAAAATATCT ATCTTCTAAACTTGGCA GTTTCTAACTTGTGTTT CTTGCTTACCCTGCCCT TCTGGGCTCATGCTG	R	KGGCGATCCCATGTG TAAAATTCTCATTGGA CTGTACTTCGTGGGC CTGTACAGTGAGACA TTTTTCAATTGCCTTC TGACTGTGCAAAGGT ACCTAGTGT	GLY-98-GLU (GLY-98-ASP)
CCRL202	AAATATAAAGGACTCAA ACGCGTGGAAAATATCT ATCTTCTAAACTTGGCA GTTTCTAACTTGTGTTT CTTGCTTACCCTGCCCT TCTGGGCTCATGCTGR	K	GGCGATCCCATGTGT AAAATTCTCATTGGAC TGTACTTCGTGGGCC TGTACAGTGAGACAT TTTTCAATTGCCTTCT GACTGTGCAAAGGTA CCTAGTGT	GLY-98 (GLY-98-ASP)
CCRL203	AACTTTTTCTCAGCCAG GAGGAGGGTGCCCTGT GGCATCATTACAAGTGT CCTGGCATGGGTAACA GCCATTCTGGCCACTTT GCCTGAAT	W	CRTGGTTTATAAACCT CAGATGGAAGACCAG AAATACAAGTGTGCA TTTAGCAGAACTCCC TTCCTGCCAGCTGAT GAGACATTCTGGAAG CATTTTCTG	TYR-167-PHE
CCRL204	AACTTTTTCTCAGCCAG GAGGAGGGTGCCCTGT GGCATCATTACAAGTGT CCTGGCATGGGTAACA GCCATTCTGGCCACTTT GCCTGAATWC	R	TGGTTTATAAACCTCA GATGGAAGACCAGAA ATACAAGTGTGCATTT AGCAGAACTCCCTTC CTGCCAGCTGATGAG ACATTCTGGAAGCAT TTTCTG	VAL-168-MET
CCRL205	TGTCCTCCCCCTATTTA TTTTTACATTTCTCTATG TGCAAATGAGAAAAACA CTAAGGTTCAGGGAGC AGAGGTATAGCCTTTTC AAGCTTGTTTTTGCC	R	TAATGGTAGTCTTCCT TCTGATGTGGGCGCC CTACAATATTGCATTT TTCCTGTCCACTTTCA AAGAACACTTCTCCC TGAGTGACTGCAAGA GCAGCTA	ILE-243-VAL
CCRL2 x1.2 A43C	TGTAAAATTCTCATTGG ACTGTACTTCGTGGGC CTGTACAGTGAGACATT TTTCAATTGCCTTCTGA CTGTGCAAAGGTACCT AGTGTTTTTGCACAAGG G	M	AACTTTTTCTCAGCCA GGAGGAGGGTGCCC TGTGGCATCATTACA AGTGTCCTGGCATGG GTAACAGCCATTCTG GCCACTTTGCCTGAA TWCRTGGTTTATA	GLY-136
CCRL2 T140G	TTTCTGGTATTTTCTCG TACTTTATCAAGACTAT GGAATCTTAGGAGACTT AACAAAAGCAAATGAGA AATTATGTTTAGAAATG TCTAACAAAATGAAT	K	CTTTGTCCTTTTAAGT ATAACACATACCTCA GGCCTCACCAGCACA TAACTACAAAAGGTT GTCCCACTTCCTTTCT GTGGCTGAGTTAGTA GAACACAG	3'UTR
CCRL2 G284A	ACATAACTACAAAAGGT TGTCCCACTTCCTTTCT GTGGCTGAGTTAGTAG AACACAGGCTCCCACC TGCCACATCAGCAGAA GGTCACCTCAACATGT GA	R	CTACCTCCCCGGAGA CCCCCCAGATCCGTA AGGATGATGCATCCT TGATCCTAAAAACATT TTCCTGTTCCTGGTG TTCAGAATTGGACTC CACACTCACT	3'UTR

Table 5: The nucleotide and amino acid sequences of the CCRL201, and CCRL202 polymorphisms

Genotype	Nucleotide Sequence	Amino acid encoded by nucleotide sequence	Frequency in UC plate
Wild type	GGG	Gly	93.2%
CCRL201	GAG	Glu	5.7%
CCRL202	GGT	Gly	1.1%

If CCRL201 and CCRL202 were to occur on the same chromosome (theoretical frequency 0.06%), the GAT sequence would encode Asp.

Example 3: Biallelic polymorphism genotyping by Pyrosequencing™

A pair of oligonucleotides for amplification by PCR was designed on either side of each biallelic polymorphism to produce a product size between 50bp and 350bp. A sequencing oligonucleotide was designed to end within 30bp either 5' or 3' to each polymorphic site. All amplification oligonucleotides used to generate the complementary strand to the sequencing primer were labelled with a 5' – Biotin.

For each marker, all samples genotyped were amplified by PCR using the PCR amplification oligonucleotides. Each reaction used: 20ng DNA (dried down), 0.6 units of AmpliTaq Gold™ DNA polymerase, 1X PCR Buffer II, 2.5mM $MgCl_2$, 1mM dNTP, and 10pmol of each PCR oligonucleotide in a final volume of 10µl. The PCR cycling conditions used were: 95°C for 12 min, 45 cycles of: 94°C for 15 sec, T_A for 15 sec (Table 6), 72°C fro 30 sec, and 72°C for 5 min.

After amplification the DNA strand of each PCR template complementary to the sequencing primer was isolated, ready for pyrosequencing (PSQ). To do this, 1) 50µl of Dynabead solution (2mg/ml Dynabeads®, 5mM Tris-HCl, 1M NaCl, 0.5 mM EDTA, 0.05% Tween 20) was added to the PCR product and shaken at 65°C for 15 min, 2) the template was transferred using magnets to 50µl of 0.5M NaOH for 1 min, 3) the template was transferred using magnets to 100µl of 1X Annealing buffer (20mM Tris-Acetate, 5mM

$MgAc_2$) for 1 min, and 4) the template was transferred using magnets to 45µl of 1X Annealing buffer containing 15pmol of sequencing oligonucleotide (Table 6).

After template isolation, the sequencing oligonucleotide was annealed to the template by denaturing at 80°C for 2min and then cooling to room temperature for 10 min. Each marker/sample combination was then sequenced/genotyped by pyrosequencing™ on a PSQ96™ (Pyrosequencing AB). Genotype results were stored in the PSQ oracle® database ready for statistical analysis.

Table 6: PSQ assay oligonucleotides and PCR annealing temperatures

Example 4: Statistical analysis of biallelic polymorphism genotyping

The five SNPs CCRL201, CCRL202, CCRL203 and CCRL204, characterised in Table 4, were analysed in a collection of simplex families ascertained through a child affected with inflammatory bowel disease. Transmission distortion to the affected child was tested using the program Transmit (Clayton. D. 1999 Am. J. Hum. Genet. 65:1170-1177). Results for ulcerative colitis are given in Table 7.

For the two SNPs CCRL202 and CCRL204, statistically significant associations of the rare allele with UC were found with p values of 0.029 and 0.013 respectively. These two SNPs were subsequently tested in an independent collection of UC cases and controls. All individuals in this analysis were self-reported Caucasians. Genotype numbers, allele frequencies and chi-squared tests for association are given in Table 8. These results replicate the findings from the earlier test for transmission distortion, namely that the rare allele at each SNP is associated with UC.

Clinical information for affected individuals in both the simplex families and the case-control cohort permitted these affected individuals to be partitioned into severe and mild cases, where severity was defined by involvement of the entire colon, surgical removal of the colon and subsequent requirement of a colostomy bag. Allele frequencies in the severe groups and among the remaining affected individuals were estimated separately and are given in Table 9. From both the simplex families and the case-control collection, the rare allele frequency in the severe group is higher. This trend is entirely consistent with the rare allele at these two SNPs increasing risk for this disease.

In order to achieve a more accurate estimate of risk for carriers of these SNPs, affected children from the TDT families, known to be Caucasian, were pooled with the cases and the pooled group compared to controls. These data, together with odds ratios and their standard errors are summarised in Table 10. These odds ratios are high for risk alleles which are themselves at high frequencies.

Table 7: Transmission distortion in Simplex families for ulcerative colitis

	CCRL201	CCRL202	CCRL203	CCRL204	CCRL205
Allele 1	A	T	A	A	G
Frequency	0.018	0.164	0.404	0.158	0.085
Observe Transmission	6	74	136	74	32
Expected Transmission	5	64	136	64	33
Allele 1	G	G	T	G	A
Frequency	0.982	0.835	0.596	0.842	0.915
Observed Transmission	348	286	204	296	324
Expected Transmission	349	296	204	308	323
Chi squared value	0.325	4.27	0.001	5.74	0.072
Probability	0.58	**0.029**	0.967	**0.013**	0.512

Table 8: Allele and genotype specific odds ratios with pooled data

	CCRL202	CCRL204
Number of genotyped cases	321	325
Number of genotyped controls	78	470
Allele 1	T	A
Frequency in cases	0.17	0.18
Frequency in controls	0.13	0.13
Allele2	G	G
Frequency in cases	0.83	0.82
Frequency in controls	0.87	0.87
Chi squared value	4.59	5.43
Probability	0.032	0.02
Genotype frequency cases		
11	13	13
12	85	89
22	223	223
Genotype frequency controls		
11	8	10
12	112	108
22	358	353
Chi squared value	4.58	4.67
Probability	0.10	0.10

Table 9: Frequency of the rare allele in severe and mild cases of ulcerative colitis

	Simplex families		Case-control cohort	
	Mild	Severe	Mild	Severe
CCRL202	0.201 (0.022)*	0.269 (0.087)	0.16 (0.017)	0.17 (0.018)
CCRL204	0.194 (0.021)	0.267 (0.081)	0.17 (0.026)	0.22 (0.029)

* (standard error)

Table 10: Allele and genotype specific odds ratios with pooled data

	CCRL202	CCRL204
Number of genotyped cases (pooled)	498	509
Number of genotyped controls	478	479
Allele 1	T	A
Frequency in cases	0.178	0.190
Frequency in controls	0.134	0.135
Allele2	G	G
Frequency in cases	0.822	0.810
Frequency in controls	0.866	0.865
Chi squared value	6.77	10.52
Probability	0.009	0.001
Odds ratio	1.4 (0.13)	1.5 (0.12
Genotype frequency cases (pooled)		
11	18	18
12	141	157
22	339	334
Genotype frequency controls		
11	8	10
12	112	109
22	358	360
Chi squared value	7.28	11.02
Probability	0.026	0.004
Odds ratio, rare homozygote	2.37 (0.43)	1.94 (0.40)
Odds ratio, heterozygote	1.33 (0.15)	1.55 (0.15)

Example 5: Expression profiling

Expression profiling by Taqman quantitative PCR

Sample sourcing

Total RNA from human tissues was purchased from a variety of commercial suppliers (BD Clontech, Stratagene, Ambion). Total RNA was prepared from cell lines (1 x 10^7 cells) using the SV total RNA isolation system (Promega) according to the manufacturer's instructions. 1ug of total RNA from a cell line or a commercial supplier (BD Clontech, Stratagene, Ambion) was reverse transcribed into first strand cDNA using the Superscript II kit (Invitrogen) according to the manufacturer's instructions. A negative RT sample was made from 1ug of total RNA in a duplicate reaction without Superscript II enzyme.

PCR assays and conditions

An 'Assay on Demand' product was ordered from Applied Biosystems to amplify CCRL2 (assay ID: Hs00243702_s1). The CCRL2 assay is not cDNA specific and a genomic RT negative control sample was also amplified to enable adjustment for any genomic contamination of samples. An internal reference 'housekeeping' gene, L32 was also amplified from the same sample set in a separate reaction to enable the quantitative PCR results for CCRL2 to be normalised for amount of input cDNA. The L32 housekeeping assay was designed using Primer Express v 2.0. The forward and reverse primer sequences were; For TCATCCGGCACCAGTCAGA, Rev TCTGGGTTTCCGCCAGTTAC. The VIC labelled Taqman probe sequence was CTT AAT TTT GAC ATA TCG.

Approximately 50ng of cDNA was used in a 20ul PCR reaction with 1x Universal Taqman master mix (Applied Biosystems). The CCRL2 assay on demand primers were added at a concentration of 900nM and the probe at 250nM (Applied Biosystems). The L32 housekeeping assay was optimised at a concentration of 200nM for the primers and 125nM of probe. Real time amplification was performed using the 7900HT thermal cycler (Applied Biosystems). This was carried out under standard conditions of 50oC for 2 mins, 95°C for 10 mins and then 40 cycles of 95°C for 15 secs and 60°C for 1 min. Data was analysed using SDSv2.0 and Microsoft Excel.

An additional experiment was carried out using a Human Digestive System cDNA panel(BD Clontech K1424-1) as a template for quantitative PCR. Approximatly 5ng of cDNA was used in a 20µl PCR reaction with 1x Universal Taqman master mix (Applied Biosystems). The CCRL2 and L32 assays were added at the optimised concentrations described previously and run on the 7900HT under standard conditions.

Results

Total RNA was extracted from human tissues and cell lines and reverse transcribed into cDNA. An assay on demand product was used to amplify CCRL2 by quantitative PCR. A genomic RT-negative control sample was also amplified to enable adjustment for any genomic contamination of samples. A housekeeping gene L32 was run to adjust for varying levels of cDNA input. The CCRL2 quantity values were extracted from the standard curve and normalised to the L32 values. Figure 3 and 5 are graphs showing the normalised quantity versus tissue type. A full list of results can be found in Table 11.

The results show a wide range of expression. The tissues and cell lines showing expression, listed in order of descending expression levels are, Monocytes, HMVECL, Ascending Colon, Lung, Small Intestine, Spleen, K562, Cervix, Thymus, Thyroid, Stomach, CCRF-CEM, U266, U937, Placenta, THP1, Heart, 28SC, SAEC, Skin, Trachea, Brain, Stimulated Jurkat, Jurkat, EoL, Ovary, Liver, Uterus, Adrenal Gland, CD3, MOLT3, Ost Day 14 + PTH, Pancreas, A443, Skeletal muscle, Testis, CD4+ and RPMI. No Expression was seen in Hela cells.

The high level of expression observed in the ascending colon sample is of particular interest as this region of the alimentary tract is subject to disease in the severe forms of ulcerative colitis. The high level of expression observed in the fresh monocyte sample confirms previous reports.

The expression results from the Human Digestive Panel indicate that CCRL2 is expressed throughout the digestive tract. These data are presented in Figure 6. The tissues showing highest expression of CCRL2 were the rectum, ascending and transverse Colon. Lower levels of expression were observed in the oseophagus, stomach, duodenum, jejunum, ileum, ileocecum, cecem descending colon and liver.

Expression profiling by microarray analysis

The probe sets TPC2078_s_at and TPC2138_s_at were selected to represent the gene CCRL2. Their sequences are shown in Table 12. These were included as two probe sets on a CustomExpress array ordered from a commercial chip provider (Affymetrix). Chips were generated using Affymetrix's standard photolithography methodology.

Hybridisation of the CustomExpress array was performed using the Affymetrix GeneChip® system. Hybridisation probes were prepared from 5ug of total RNA according to manufacturers instructions. 5ug of fragmented probe was hybridised to an Affymetrix Test3 array at 45^0C for 16 hours. Array washing and probe detection was performed using the manufacturers standard protocols (Affymetrix). The Test3 arrays were scanned with the Agilent Gene Array scanner and data extracted using the MAS5 software (Affymetrix). Test array QC was performed according to the manufacturer's instructions. For probes passing test array QC, 10ug were hybridised to the CustomExpress array at 45^0C for 16 hours. Hybridisation, washing and detection conditions were performed according to manufacturers provided protocols The expression data was extracted using MAS5 software (Affymetrix) to a target value of 100.

Signal and detection calls were plotted using Microsoft Excel to visualise expressio profiles of CCRL2.

Results

A custom Affymetrix array was manufactured with the probe sets TPC2138_s_at and TPC2078_s_at representing CCRL2. The arrays were hybridised with cRNA from 28 tissues and cell lines. Five tissues have been duplicated using RNA from an alternative source. Figure 5 graph shows the signal for each probe type versus tissue type with the call highlighted by colour. Present calls are white, marginal are grey and absent are black. All the results are summarised in Table 13 the signal, P value and call (present or absent) for each tissue type.

Present calls were seen for, Thymus, Ascending colon, Small intestine, Stomach, Thyroid, Lung, Cervix, Skin, 28SC P7, U937 Monocytes and fresh Monocytes, suggesting that CCRL2 is highly expressed in these tissues. Marginal calls were seen for Heart, Stimulated Jurkat and Ovary. The data for the two probe sets were generally consistent. The exceptions were TPC2138_s_at is called absent in Skin and TPC2078_at is present; TPC2138_s_at is called marginal in Stomach and TPC207_at is present; TPC2078_at being

marginal in Heart (pooled) and TPC2138_s_at is absent;TPC2078_at is called marginal in Ovary and TPC2138_s_at is absent; TPC2078_at is called present in 28SC and TPC2138_s_at is absent; TPC2078_at is called marginal in Stimulated Jurkat and TPC2138_s_at is absent; TPC2078_at is called present in U937 Monocytes TPC2138_s_at is marginal.

These results are comparable with the Taqman results with the positive calls, Thymus, Ascending colon, Small intestine, Stomach, Thyroid, Lung, Cervix, Skin, 28SC P7, U937 Monocytes and fresh Monocytes all showing a high level of expression. The Taqman results have suggested that expression is seen in a wider range of tissue than the array results. This is likely due to the fact that the Taqman technology is more sensitive and can detect a lower level of expression than the arrays.

Table 11. Taqman results

Tissue	Normalised Quantity
28SC	0.000632443
THP1	0.003068319
HMVECL	0.019699581
U266	0.003704836
EoL	0.001167639
CCRF-CEM	0.003784275
K562	0.008634736
HEK 293	4.02903E-05
MOLT3	0.000763577
A431	0.000466955
Jurkat	0.00132302
CACO-2	-0.000616877
NHEK	-0.00017836
Raji-B	2.37783E-05
Hela	0
U937	0.003267471
SAEC	0.0025231
RPMI	0.000185122
Skeletal muscle	0.000451236
Trachea	0.002365127
Brain	0.002340819
Skin	0.002460081
Thymus	0.007985788
Spleen	0.009055845
Pancreas	0.000562676
Ascending Colon	0.016652544
Uterus	0.001110581
Small Intestine	0.010308762
Stomach	0.004491638
HMVECL	0.005945802
Heart	0.002839997
Adrenal Gland	0.000971981
Thyroid	0.006587376
SAEC	0.001262788
Liver	0.001233166
Lung	0.016172315
Cervix	0.008565696
REF	0.001652083
Placenta	0.00312852
Testis	0.000268765
Ovary	0.001257188
28SC	0.002657724
CD3	0.000899092
CD4+	0.000220043
EoL	0.001262948
Jurkat	0.000700008
Stimulated Jurkat	0.001329283
NHEK	3.59159E-05
Monocytes	0.028760903
Raji B cells	8.90792E-06
Ost Day 14	-0.00067786
Ost Day 14 + PTH	0.000714696
THP1	0.002444509

Table 12. Sequences of oligonucleotide probe sets used to identify CCRL2 expression by hybridisation.

Probe	Sequence
TPC2078_s_at	ACCTGACCATTCCACCGAAGTGTAA
	CTTTACAAACGTGAGCTCCTTCGCC
	CATTTGCAAATACCTCTGCCGCTG
	CTCTCCTGTATGCGTTTCTTGATGG
	CTCAGGCACCGTGCAAGGCTCTTTA
	CCTCCTACCACTTGTCCATAGTGTG
	GCCTACGCTTGGTCCAGAACATCAA
	GATTTTCATCTTTCTGCATTATTTC
	GTTCACATCACTAAACTCATCGCCA
	TGATTCTCCAGCCCTGGTAGCATTT
	TTCAACCCAGGGGGCAGTCTGCACA
TPC2138_s_at	ACCTGACCATTCCACCGAAGTGTAA
	CTTTACAAACGTGAGCTCCTTCGCC
	CATTTAGCAAATACCTCTGCCGCTG
	CTCTCCTGTATGCGTTTCTTGATGG
	CTCAGGCACCGTGCAAGGCTCTTTA
	CCTCCTACCACTTGTCCATAGTGTG
	GCCTACGCTTGGTCCAGAACATCAA
	GATTTTCATCTTTCTGCATTATTTC
	GTTCACATCACTAAACTCATCGCCA
	TGATTCTCCAGCCCTGGTAGCATTT
	TTCAACCCAGGGGGCAGTCTGCACA

Table 13: Affymetrix results

Tissue	Detection TPC2138_s_at	Detection TPC2078_s_at	Signal TPC2138_s_at	Signal TPC2078_s_at	P-value TPC2138_s_at	P-value TPC2078_s_at
Skeletal Muscle	Absent	Absent	199	202.3	0.19458	0.11157
Trachea	Absent	Absent	174.8	197.4	0.21948	0.14966
Skin (Pool 2)	Absent	Absent	192.6	248.2	0.09522	0.06763
Skin (Single)	Absent	Present	142.5	233.1	0.12964	0.03027
Thymus	Present	Present	285.6	315.5	0.03027	0.00806
Pancreas	Absent	Absent	158.7	151.2	0.19458	0.30371
Ascending Colon	Present	Present	489.5	382.4	0.00586	0.00293
Uterus	Absent	Absent	255.9	262.2	0.08057	0.09522
Small Intestine	Present	Present	394.4	403.9	0.02393	0.01856
Stomach	Marginal	Present	303.4	332.2	0.05615	0.02393
Heart (Single)	Absent	Absent	267.5	216.3	0.14966	0.06763
Heart (Pool 3)	Absent	Marginal	103	310.6	0.08057	0.05615
Adrenal Gland	Absent	Absent	172	225.4	0.14966	0.12964
Thyroid	Present	Present	266.1	391.1	0.03027	0.01074
Liver	Absent	Absent	147.9	239.8	0.27417	0.06763
Lung (Pool 2)	Present	Present	537.6	547.8	0.00195	0.00195
Lung (Single)	Present	Present	464	392.5	0.01416	0.01074
Cervix (Pool 6 A)	Present	Present	272.3	315.6	0.0376	0.03027
Cervix (Pool 6 B)	Absent	Absent	178.7	208.3	0.19458	0.06763
Placenta	Absent	Absent	243	286	0.11157	0.09522
Testis	Absent	Absent	94.9	112.4	0.21948	0.21948
Ovary (Single B)	Absent	Absent	124.8	136.2	0.21948	0.14966
Ovary (Single A)	Absent	Marginal	227.7	271.3	0.09522	0.05615
28SC P7	Absent	Present	166.9	311.9	0.08057	0.03027
CD4+	Absent	Absent	217.9	192.7	0.24609	0.17139
EOL-1	Absent	Absent	109.5	125	0.36621	0.19458
Jurkat	Absent	Absent	52.7	121	0.39893	0.21948
Jurkat Stimulated	Absent	Marginal	148.1	295.7	0.11157	0.05615
NHEKB	Absent	Absent	265.7	142.2	0.08057	0.24609
Monocytes	Present	Present	612.6	614.4	0.00293	0.00293
Osteoblasts day 14	Absent	Absent	83.4	218.5	0.24609	0.21948
Osteoblasts day 14 + PTH	Absent	Absent	143.3	118.9	0.21948	0.21948
HEK293	Absent	Absent	165.5	247.9	0.24609	0.17139
U937 Monocytes	Marginal	Present	263.9	265.2	0.05615	0.04614

CLAIMS

1. A method of determining whether an individual is predisposed to inflammatory bowel disease, which method comprises identifying whether the individual has a polymorphism in the CCRL2 polynucleotide or protein which polymorphism is associated with inflammatory bowel disease.

2. A method according to claim 1 comprising detecting (i) whether the individual has a one or more of the polymorphisms defined in Table 4, and/or (ii) a polymorphism which is in linkage disequilibrium with any of the polymorphisms defined in Table 4.

3. A method according to claim 1 or claim 2, wherein inflammatory bowe disease is ulcerative colitis.

4. A method according to any one of claims 1 to 3 which comprises contacting a sample from the individual with a specific binding agent for the polymorphism and determining whether the agent binds to the polymorphism, wherein binding of the agent to the polymorphism indicates that the individual is predisposed to inflammatory bowel disease.

5. A method according to any one of the preceding claims wherein the polymorphism in the CCRL2 polynucleotide is determined for both alleles of the individual.

6. A method for preventing or treating an individual who has been identified as having a predisposition to inflammatory bowel disease by a method as defined in any one of the preceding claims, comprising administering to the individual a therapeutically effective amount of an agent which prevents or treats inflammatory bowel disease.

7. Use of an agent which prevents or treats inflammatory bowel disease, in the manufacture of a medicament for use in preventing treating an individual, which

individual has been identified as having a predisposition to inflammatory bowel disease, using a method according to any one of claims 1 to 5.

8. A method for identifying an agent for the prevention or treatment of inflammatory bowel disease, which method comprises determining whether a test agent is capable of (i) binding a CCRL2 polypeptide or polynucleotide, or (ii) modulating the expression or activity of a CCRL2 polypeptide or polynucleotide.

9. A method for identifying an agent for the prevention or treatment of inflammatory bowel disease, which method comprises:

(i) contacting a test agent with a CCRL2 polypeptide or polynucleotide; and

(ii) determining whether the test agent is capable of binding to the polypeptide or polynucleotide and modulating the activity or expression of the polypeptide or polynucleotide.

10. A method according to claim 9 wherein:

(i) the CCRL2 polypeptide or polynucleotide is a fragment and/or homologue of the sequence shown in SEQ ID NO:1 or 2, and/or

(ii) the CCRL2 polypeptide and/or polynucleotide has a mutation or polymorphism associated with inflammatory bowel disease

11. A method of formulating a pharmaceutical composition comprising:

(i) identifying an agent for the prevention or treatment of inflammatory bowel disease by a method as defined in claim 8; and

(ii) formulating the agent with a pharmaceutically acceptable carrier or diluent.

12. An agent identified by the method of any one of claims 8 to 10.

13. A pharmaceutical composition for the prevention or treatment of inflammatory bowel disease comprising an agent according to claim 12 and a pharmaceutically acceptable carrier or diluent.

14. Use of an agent according to claim 12 in the manufacture of a medicament for use in treating or preventing inflammatory bowel disease.

15. A method of preventing or treating inflammatory bowel disease, which method comprises administering a therapeutically effective amount of an agent according to claim 12 to an individual in need thereof.

16. A method of treating inflammatory bowel disease, which method comprises:

(i) identifying an agent for the prevention or treatment of inflammatory bowel disease by a method as defined in claim 10; and

(ii) administering a therapeutically effective amount of an agent identified in (i) to an individual having inflammatory bowel disease.

17. Use of a CCRL2 polynucleotide or polypeptide, not having a mutation or polymorphism associated with inflammatory bowel disease, in the manufacture of a medicament for the prevention or treatment of inflammatory bowel disease.

18. A probe, primer or antibody for use in a method according to any one of claims 1 to 5 which is capable of selectively detecting a polymorphism in the CCRL2 polynucleotide or protein which is associated with inflammatory bowel disease.

19. An antibody capable of selectively binding to a polymorphism associated with inflammatory bowel disease in the CCRL2 protein.

20. A method of identifying a polymorphism which can be used to determine whether an individual has a genetic predisposition to inflammatory bowel disease, the method comprising screening the CCRL2 polynucleotide or polypeptide of one or more individuals.

21. A method for treating or preventing inflammatory bowel disease in an

individual comprising:

(i) identifying, in the individual, the presence of a polymorphism in the CCRL2 polynucleotide or CCRL2 protein which is associated with inflammatory bowel disease; and

(ii) introducing into the individual a CCRL2 polynucleotide of the invention which is a different allele of the polymorphism or polymorphisms to that present in the individual.

22. A cell line comprising a CCRL2 polynucleotide having a polymorphism associated with inflammatory bowel disease.

23. A non-human animal which is transgenic for a CCRL2 polynucleotide having a polymorphism associated with inflammatory bowel disease.

24. Use of a cell line according to claim 22 or non-human animal according to claim 21 in screening for an agent for use in diagnosis, prevention or treatment of an individual having a genetic predisposition to inflammatory bowel disease.

25. A method for determining the efficacy of an agent useful in the treatment of inflammatory bowel disease in an individual having a polymorphism associated with inflammatory bowel disease in the CCRL2 polynucleotide, which method comprises:

(i) providing a CCRL2 polynucleotide having said polymorphism, or a protein encoded thereby;

(ii) contacting said protein or gene with said agent; and

(iii) determining whether said protein or gene interacts with said agent.

26. A method according to claim 25, wherein said gene or protein is in a subject having said polymorphism or in a non-human animal according to claim 20 and step (iii) comprises monitoring the effect of administering the agent on one or more phenotype of inflammatory bowel disease.

27. An apparatus arranged to perform a method according to any one of claims 1 to 6 comprising:

(i) means for receiving genetic information;

(ii) a module for comparing the genetic information with a database comprising information relating to a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease; and

(iii) means for determining on the basis of said comparison the susceptibility of the individual to inflammatory bowel disease.

28. A computer program comprising program code means that, when executed on a computer system, instructs the computer system to perform a method according to any one of claims 1 to 6.

29. A computer program product comprising a computer-readable storage medium having recorded thereon a computer program according to claim 28.

30. A database comprising information relating to a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease.

31. A computer program product comprising program code means on a carrier wave, which program code means, when executed on a computer system, instruct the computer system to perform a method according to any one claims 1 to 6.

32. Method for determining whether or not an individual is predisposed to inflammatory bowel disease, which method comprises:

(i) inputting genetic data from said individual to a computer system; comparing said genetic data to a computer database, which database comprises information relating to a polymorphism in the CCRL2 polynucleotide or protein, which polymorphism is associated with inflammatory bowel disease; and

(ii) determining on the basis of said comparison whether said individual has said polymorphism thereby determining whether the individual is predisposed to inflammatory bowel disease.

Figure 1

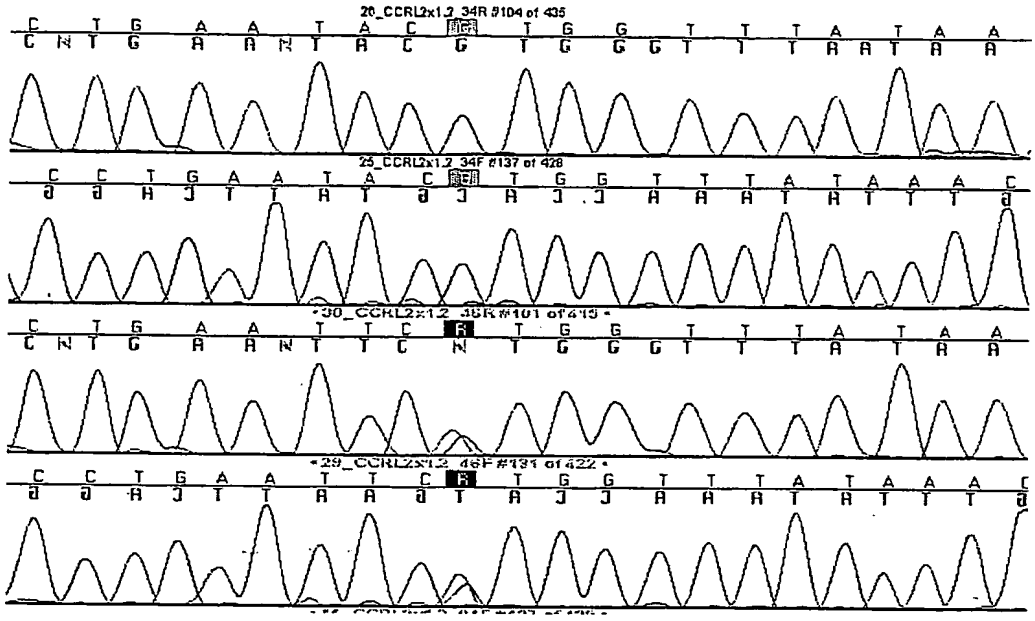
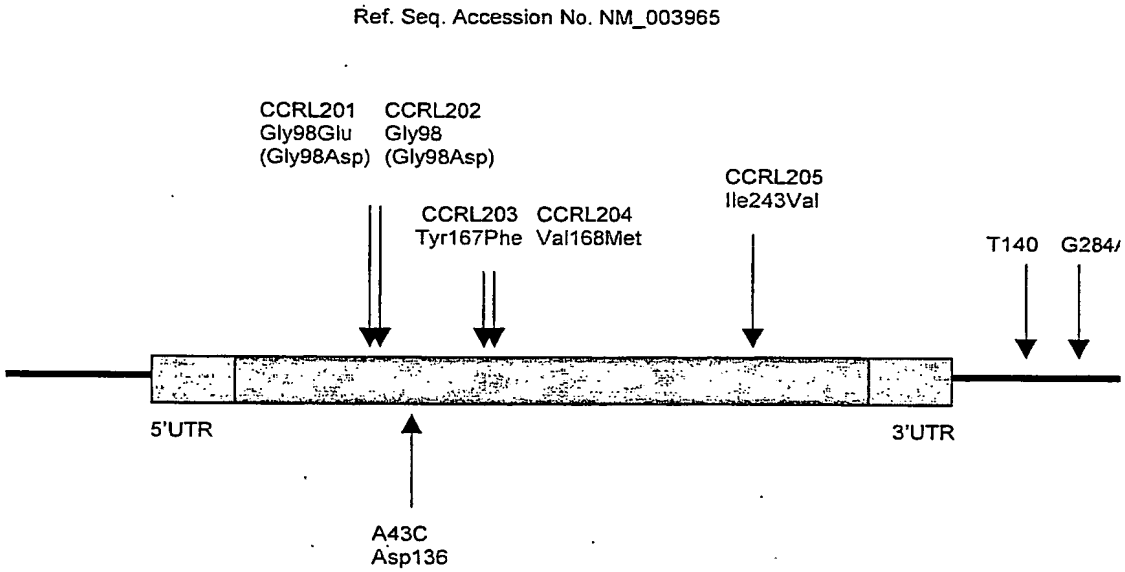
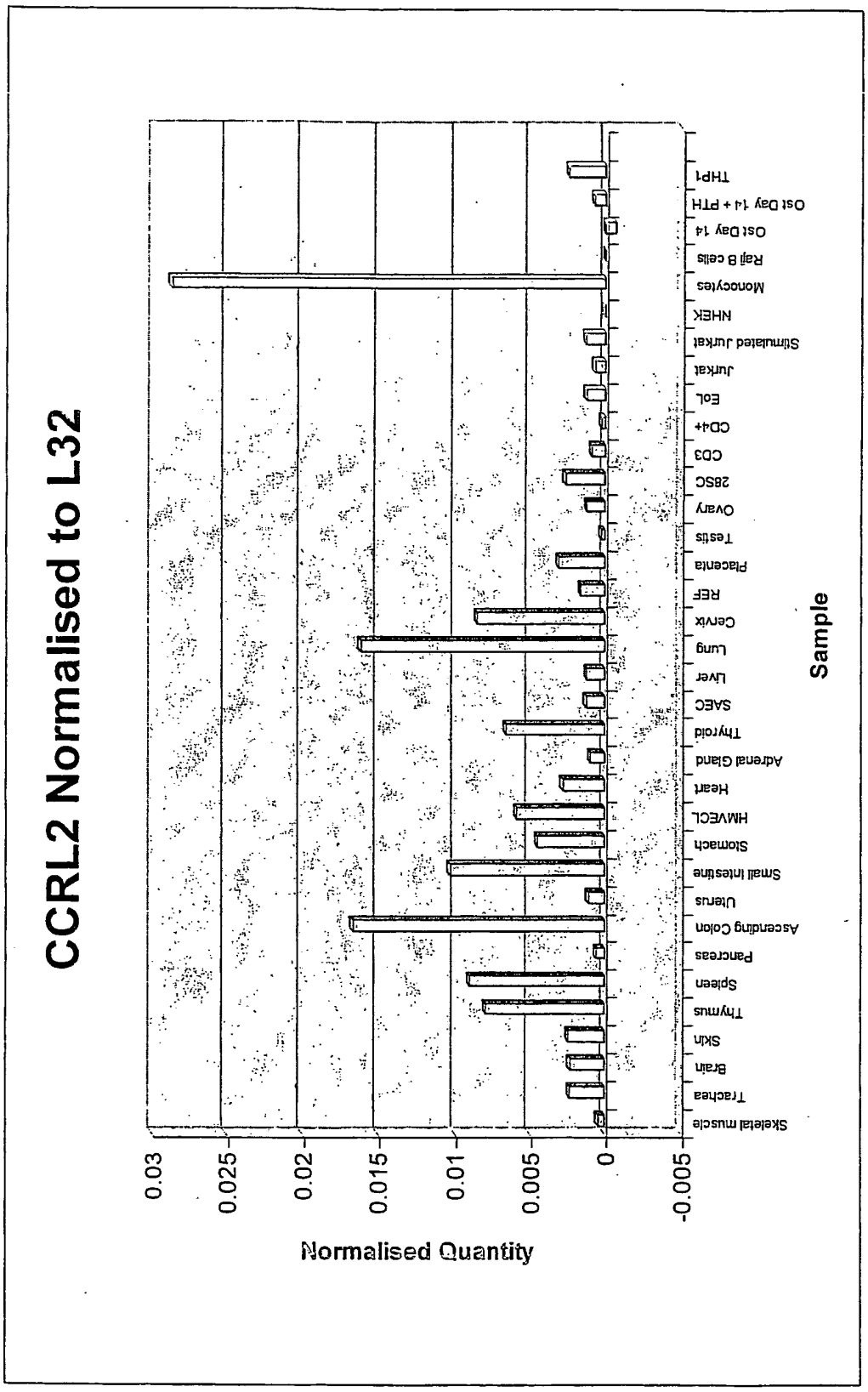


Figure 2





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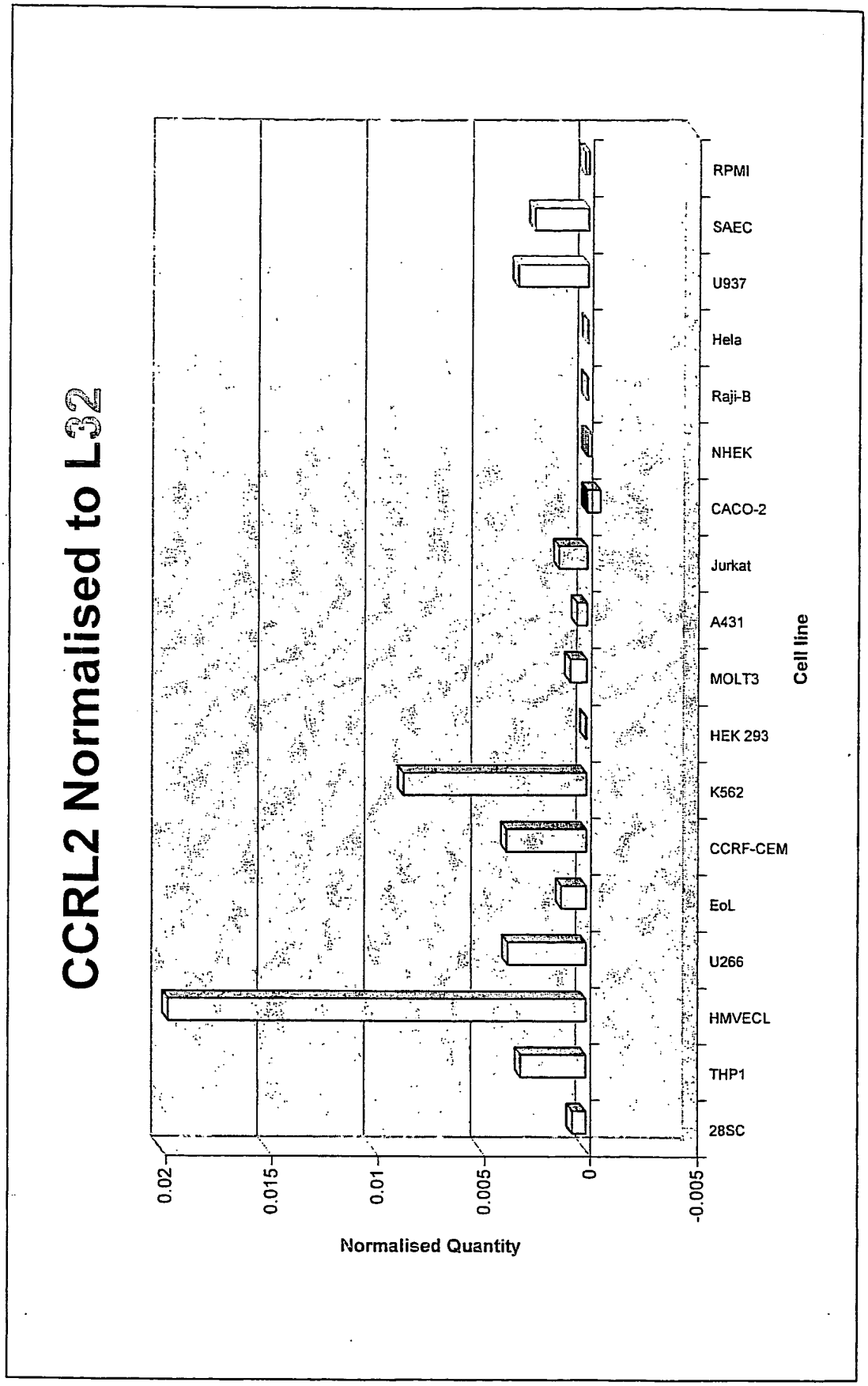

Figure 4

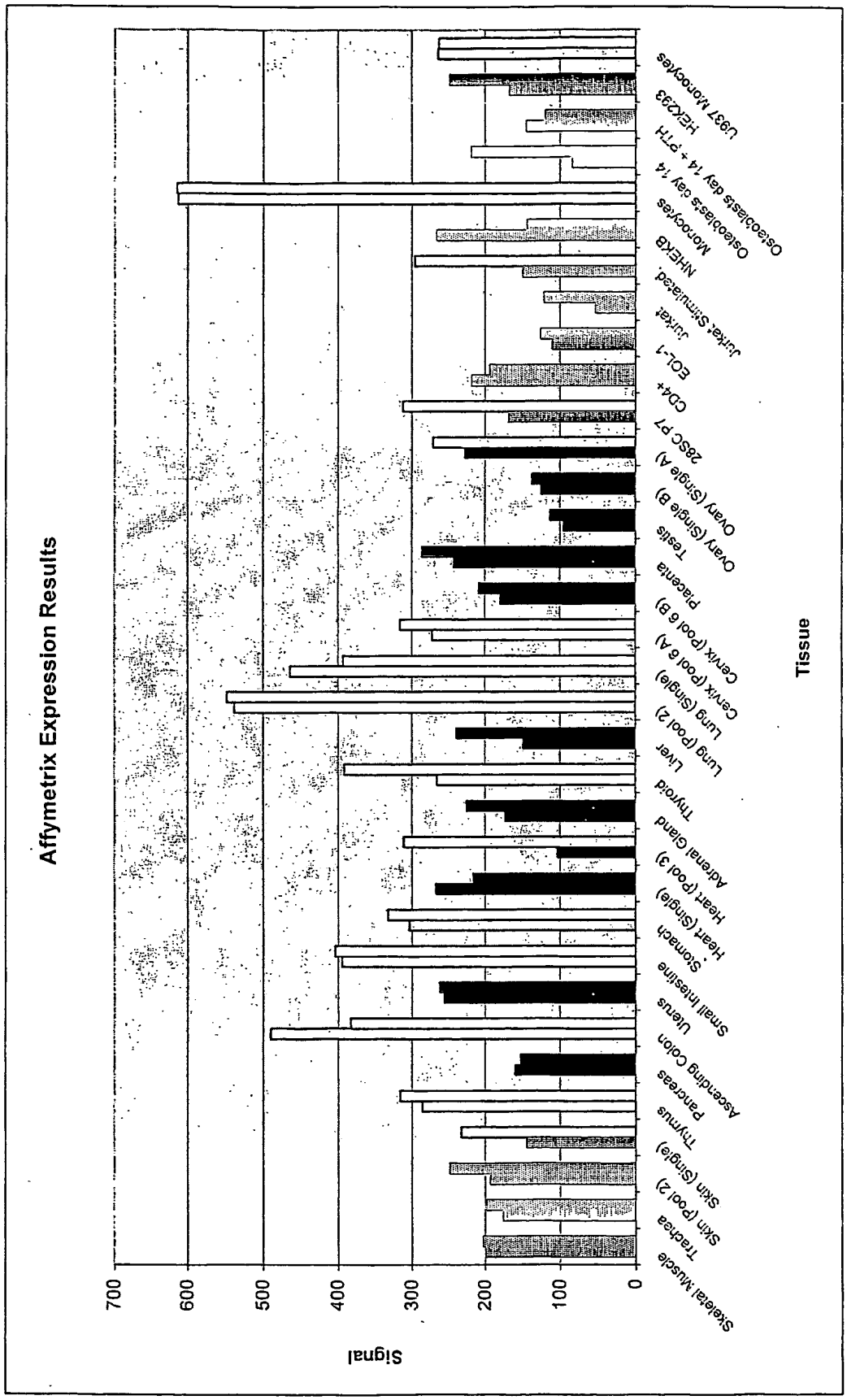

Figure 5

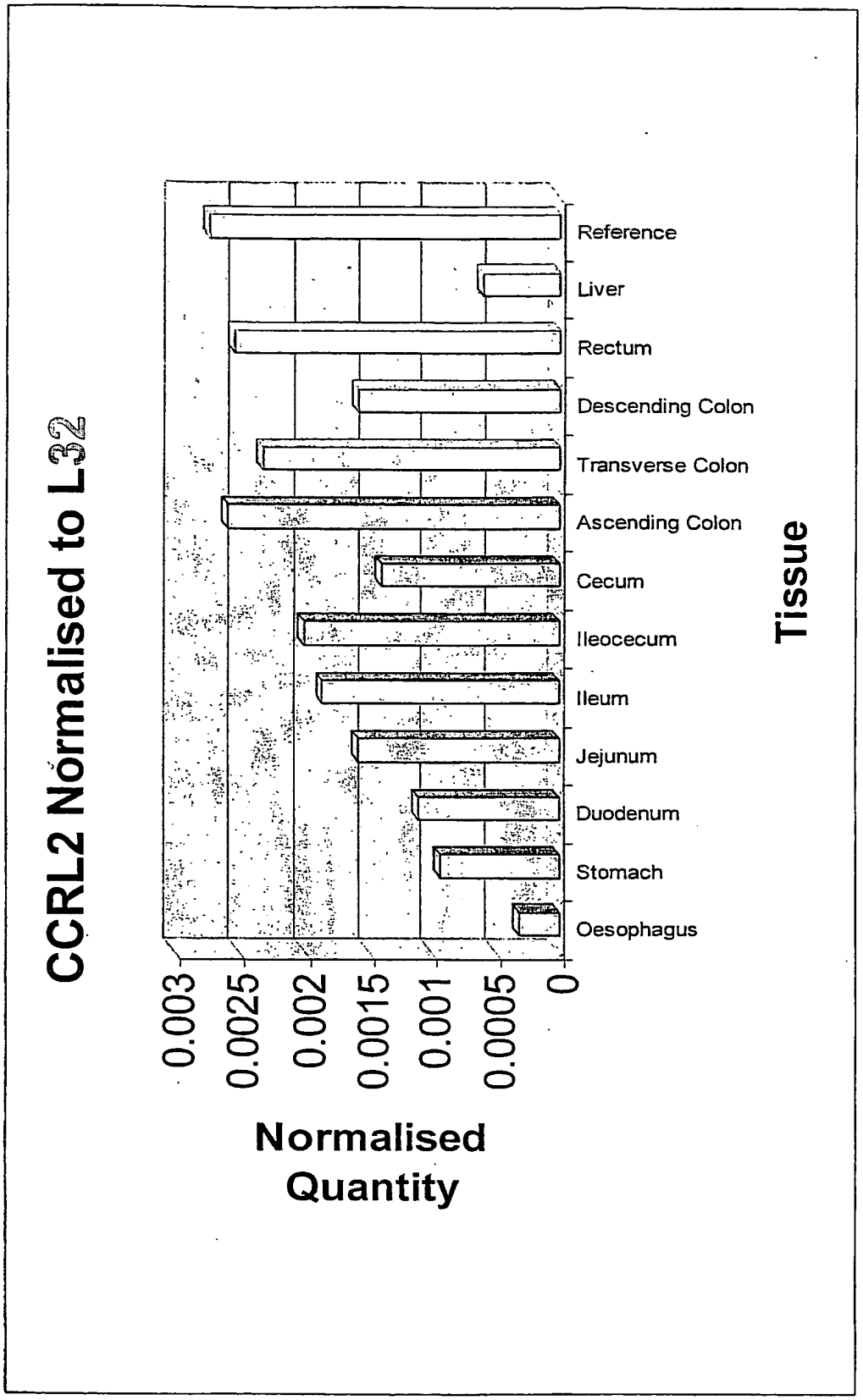

Figure 6

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Ala Val Ser Asn Leu Cys Phe Leu Leu Thr Leu Pro Phe Trp Ala His
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Glu Pro Asp His Ser Thr Glu Val
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
20 January 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RECEPTOR PROTEINS

(57) Abstract: A method of determining whether an individual is predisposed to inflammatory bowel disease, which method comprises identifying whether the individual has a polymorphism in the CCRL2 polynucleotide or protein which polymorphism is associated with inflammatory bowel disease.

WO 2004/083232 A3

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/GB2004/001159

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/061087 A (ROUSH CHRISTINE L ; BROWN JOSEPH P (US); BURMER GLENNA C (US); LIFESPA) 8 August 2002 (2002-08-08) the whole document	1
A	WO 01/46698 A (TALBOT DALE ; WEI ZHENG (US); MIAO ZHENHUA (US); SCHALL THOMAS J (US);) 28 June 2001 (2001-06-28) the whole document	1
A	WO 98/39441 A (INCYTE PHARMA INC ; AU YOUNG JANICE (US); CHENG MUZONG (US); GUEGLER K) 11 September 1998 (1998-09-11) the whole document	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2004	15/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Gabriels, J

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

mational application No.

PCT/GB2004/001159

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [X] in computer readable form

   c. time of filing/furnishing

   [ ] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [X] furnished subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB2004/001159

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 6,15,16,21,26,28-32
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(vi) PCT - Program for computers
Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

2. [X] Claims Nos.: 7, 11-14,17,27
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark: on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 6,15,16,21,26,28-32

Rule 39.1(vi) PCT - Program for computers
Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

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Continuation of Box II.2

Claims Nos.: 7, 11-14,17,27

Present claim 27 relates to an apparatus defined by reference to a desirable characteristic or property, namely being able to perform the method according to any of claims 1 to 6.
The claim covers all apparatus having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for non of such apparatus. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the apparatus by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, no search has been carried out for claim 27.

Present claims 7, 11-14, and 17 relate to products and compounds defined by reference to a desirable characteristic or property, namely. being able to prevent or treat inflammatory bowel disease.
The claims cover all products and compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for non of such products and compounds . In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the products and compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, no search has been carried out for claims 7, 12-14, and 17

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Int. al Application No
PC.../US2004/001159

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02061087 A	08-08-2002	WO 02061087 A2	08-08-2002
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